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In Vitro Colony Interactions Among Species of *Trichoderma* With Inference Toward Biological Control

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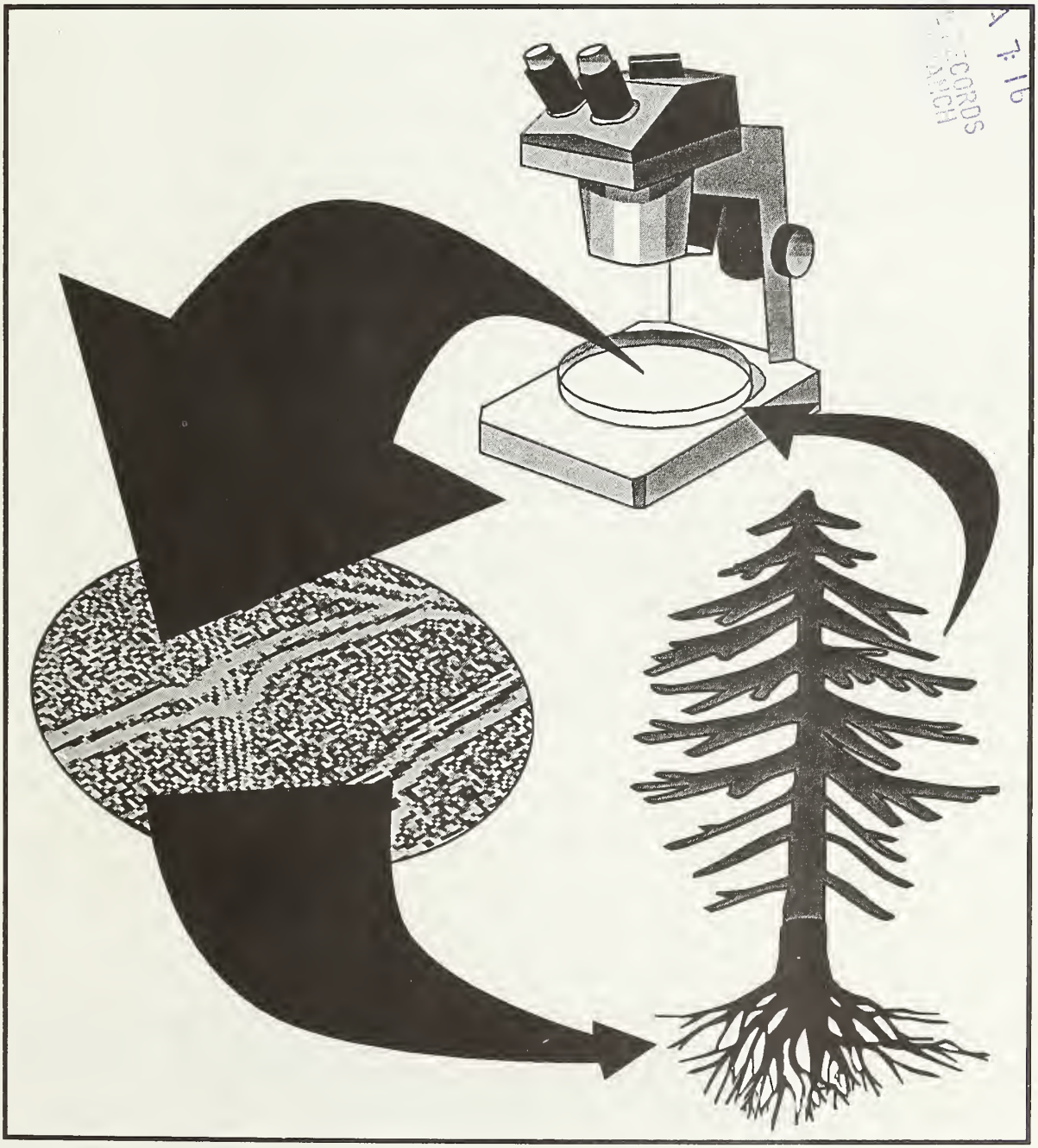

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Abstract

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Colony interactions among 15 isolates representing seven species of *Trichoderma* were evaluated *in vitro.* Interactions characterized by zones of inhibition, demarcation lines, ridges of conidia, overgrowth, intermingling, anastomosis, and hyphal coiling in self-pairings and intraspecific and interspecific pairings of the seven species were recorded. All types of interactions occurred in interspecific pairings except intermingling in dual culture. Intermingling interactions were most common in self-pairings. Demarcation lines and overgrowth interactions were absent in self-pairings and intraspecific pairings. Microscopic examination of *Trichoderma* hyphae in the interaction zone between colonies revealed that anastomosis occurred only in self-pairings and intraspecific pairings, whereas circular and lateral coiling occurred in all pairing types. Terminal and intercalary chlamydospores formed in the interaction zones in all pairings. An understanding of the compatibility between species or isolates of *Trichoderma* will provide information on the use of multiple species of *Trichoderma* as biological control agents.

Keywords: *Trichoderma* spp., biological control, antagonism.

Summary

The use of *Trichoderma* species and other organisms as biological control agents is promising. The inability of *Trichoderma* species to establish and proliferate in various substrates, however, remains a major obstacle for successful biological control. Some parts of this reduced vigor is due to inability to overcome competition by indigenous microorganisms in various substrates.

Antagonism occurred in self-pairings and intraspecific and interspecific pairings of *Trichoderma* on solid medium. However, antagonism was more prevalent in interspecific pairings. Similarly, in microscopic examinations, antagonistic characteristics such as terminal and intercalary chlamydospores and circular coiling occurred only in intraspecific and interspecific pairings. Therefore, *in vitro* antagonism studies and microscopic examination should be considered in the evaluation of *Trichoderma* species as potential biological control agents. An understanding of the compatibility among species or isolates of *Trichoderma* under various cultural conditions will provide information on the use of multiple species of *Trichoderma* as biological agents against a particular plant pathogen.

Introduction

The use of *Trichoderma* species and other organisms as biological control agents is promising. The inability of *Trichoderma* species to establish and proliferate in various substrates, however, remains a major obstacle for successful biological control (Alexander 1971, Boosalis and Mankau 1970, Lewis and Papavizas 1984). Some parts of this reduced vigor is due to inability to overcome competition by indigenous microorganisms in various substrates (Papavizas 1985).

Several studies report on competitive ability among species of *Trichoderma* and between *Trichoderma* and pathogenic fungi based on antibiotic production, enzyme production, or substrate colonization (Widden 1984, Widden and Abitbol 1980, Widden and Hsu 1987, Widden and Scattolin 1988). Such incompatibility or antagonism by other organisms or indigenous populations of *Trichoderma* may limit the effectiveness of a particular species or a combination of species of *Trichoderma* as biological control agents. For example, linear growth rate and the antagonistic ability of *Trichoderma* towards *Phellinus weirii* (Murr.) Gilb., a root pathogen, in culture was affected over a range of incubation temperatures (Goldfarb and others 1989). In another study, Tronsmo and Dennis (1978) report a similar relation between temperature and antagonism potential for several species of *Trichoderma*. These reports suggest that a combination of more than one species or isolate of *Trichoderma* should be used to compensate for temperature fluctuations in woody or soil substrates, thus providing continued antagonism during each season of the year.

The objective of this study was to determine the extent of antagonism among species of *Trichoderma* in self-pairings and intraspecific and interspecific pairings *in vitro*. This information could be useful in selecting appropriate species and isolate combinations of *Trichoderma* to be used against target pathogens.

Materials and Methods

Two isolates each of *Trichoderma polysporum* (Link ex Pers.) Rifai, *T. hamatum* (Bon) Bain., *T. citrinoviride* Bis., *T. saturnisporum* Hamill, and *T. viride* Per. ex Fr. and one isolate of *T. harzianum* Rifai and *T. longibrachiatum* Rifai were isolated from Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) stumps colonized by *Phellinus weirii* in Columbia County, Oregon, in 1980 (Nelson and others 1987). A second isolate of *T. harzianum* was obtained from Dr. Earl Nelson, Forestry Sciences Laboratory, Corvallis, Oregon. Two isolates of each species, except for the single isolate of *T. longibrachiatum*, were used. All pairings were conducted in plastic petri dishes (90 by 15 millimeters) on malt agar (MA) (Difco,[1] 45 grams per liter of distilled water). For microscopic examination, malt extract broth (ME) (20 grams per liter of distilled water) was used. All pairings were conducted at 25 °C under continuous fluorescent light.

Three replicate pairings of each isolate were made, including self-paired controls, interspecific pairings, and intraspecific pairings in the dual culture experiment on MA. The number of times a given interaction occurred was recorded. One observation per pairing was made in the microscopic experiment on ME.

[1] The use of trade or firm names in this publication is for reader information and does not imply endorsement by the U.S. Department of Agriculture of any product or service.

Mycelial discs of each *Trichoderma* isolate were placed 8.5 centimeters apart on petri dishes containing MA and incubated for 15 days at 25 °C, after which colony interactions were recorded. The following interaction types were recorded for *Trichoderma* in dual culture: (1) zone of inhibition—area with no mycelium between approaching colonies (fig. 1); (2) intermingling—mycelia merged between colonies (Sharland and Rayner 1986) (figs. 2 and 3); (3) demarcation line—a submerged pigmented line formed within the medium between colonies; (4) ridge of conidia—abundant conidia formed where the mycelia of colonies met (fig. 4); and (5) overgrowth—one colony overgrew the other and sporulated (Goldfarb and others 1989) (fig. 5).

Microscopic examinations were made of all pairings in the following manner. Sterilized microscope slides were laid on surface of water agar in petri dishes. For pairings, a 1-millimeter mycelial disc of a *Trichoderma* isolate was placed at each end of a slide. A 0.5-milliliter aliquot of ME was then placed onto each microscope slide and spread evenly between the discs with a sterile glass rod. Those *Trichoderma* spp. that grew considerably slower than others were placed on the slide 2 to 4 days with ME before inoculation with the faster growing isolates (*T. polysporum* only). The slides with the *Trichoderma* isolates and ME were then placed in petri dishes on the surface of water agar and covered. The inoculated slides were incubated at 25 °C in the dark until the hyphae of the isolates converged. For controls, a single isolate of *Trichoderma* was placed at one end of a slide and incubated in the same manner. Microscope slides were removed from petri dishes, a drop of lactic acid (85 percent) was placed on the slide, and a cover slip set in place. The hyphal interactions were photographed through a Zeiss phase-contrast microscope. The following interactions were observed: (1) circular coiling—two or more hyphae of the same or different isolates encircle one another (fig. 6); (2) lateral coiling—hyphae of the same or different isolates wrapped around one another to form a helixlike structure (fig. 7); (3) anastomosis—fusion between branches of the same or different hyphae to make a network (Ainsworth 1971) (fig. 8); and (4) unaffected hyphae—hyphae grew without any coiling or anastomosis.

Results

In self-pairings of *Trichoderma* species and isolates, intermingling occurred most frequently (69 percent) followed by zones of inhibition (23 percent). Ridges of conidia occurred less often (7 percent). There were no demarcation lines or overgrowths present in self-pairings.

In intraspecific pairings, intermingling occurred most commonly, but in some cases, ridges of conidia or zones of inhibition formed. There were no demarcation lines or overgrowths present in the intraspecific pairings (table 1).

In interspecific pairings, ridges of conidia were abundant and demarcation lines common. Intermingling did not occur in interspecific pairings (table 1).

Anastomosis was the most frequently observed response in self-pairings and intraspecific pairings (table 2). There also was circular coiling between hyphae in some interaction zones (fig. 6). Otherwise, the hyphae grew along side and across each other and appeared unaffected by one another.

In interspecific pairings, however, anastomoses were not observed. Circular hyphal coiling occurred frequently (fig. 6), whereas lateral coiling (fig. 7) occurred sporadically (table 2). Anastomoses occurred in self-pairings of all species and isolates of *T. citrinoviride* (fig. 8). Terminal (Tc) and intercalary (Ic) chlamydospores similar to those observed by Papavizas and others (1984) formed in all pairings when either isolates of *T. citrinoviride* and *T. harzianum* or one of the two isolates of *T. hamatum* were present (figs. 9 and 10). Coiling (lateral or circular) or formation of chlamydospores, or both, occurred only in the interaction zones between paired colonies and not in other portions of the colony away from the interaction zone or in single colonies.

Discussion

It was surprising that zones of inhibition occurred at such a high frequency in self-pairings (that is, autoinhibition). Zones of inhibition can be caused by diffusion of toxic metabolite(s) in advance of hyphae (Dennis and Webster 1971). Because the inoculum was taken from the same mother colony, more intermingling interactions were expected in these dual cultures.

Some species that produced zones of inhibition in self-pairings, however, are strongly antagonistic to pathogenic fungi in culture (Reaves and others 1990); and the antagonism was caused by the release of inhibitory metabolites into the agar medium. Widden (1984) suggests that antibiotics are unlikely to play a role in the competitive ability among species of *Trichoderma*, because they produce similar kinds of antibiotics. In contrast, Bruce and others (1984) report that when fungal mycelia growing proximate to each other occupy the same substrate, volatile inhibitors produced by one occupant may have a significant effect on the other(s).

The data presented in this study suggest that the zones of inhibition seen in self-pairings may have resulted from high quantities of inhibitory metabolites produced by one or both daughter colonies growing in dual culture, thus causing autoinhibition. Also, because a rich medium was used and the *Trichoderma* isolates were incubated for a short period, it is unlikely that staling products, which may accumulate in the medium, caused zones of inhibition to occur.

The high incidence of ridges of conidia in interspecific pairings may be indicative of a triggered response by each isolate to produce an abundance of conidia when physical contact is made between hyphae of different species of *Trichoderma*. This type of response was abundant when four of the seven *Trichoderma* species were grown in dual culture with fungi isolated from coarse woody debris or nursery soils.[2] The low frequency of ridges of conidia in self-pairings and intraspecific pairings may be related to the ability of these isolates to anastomose and exchange genetic material. This was evident from the high frequency of intermingling interactions in dual culture and the profuse anastomoses observed in microscopic examinations in self-pairings and intraspecific pairings. If in fact, as suggested by Rifai (1969) and Bissett (1984), some species of *Trichoderma* are likely to be aggregates rather than genetically distinct species, then intermingling in interspecific and intraspecific pairings could possibly occur.

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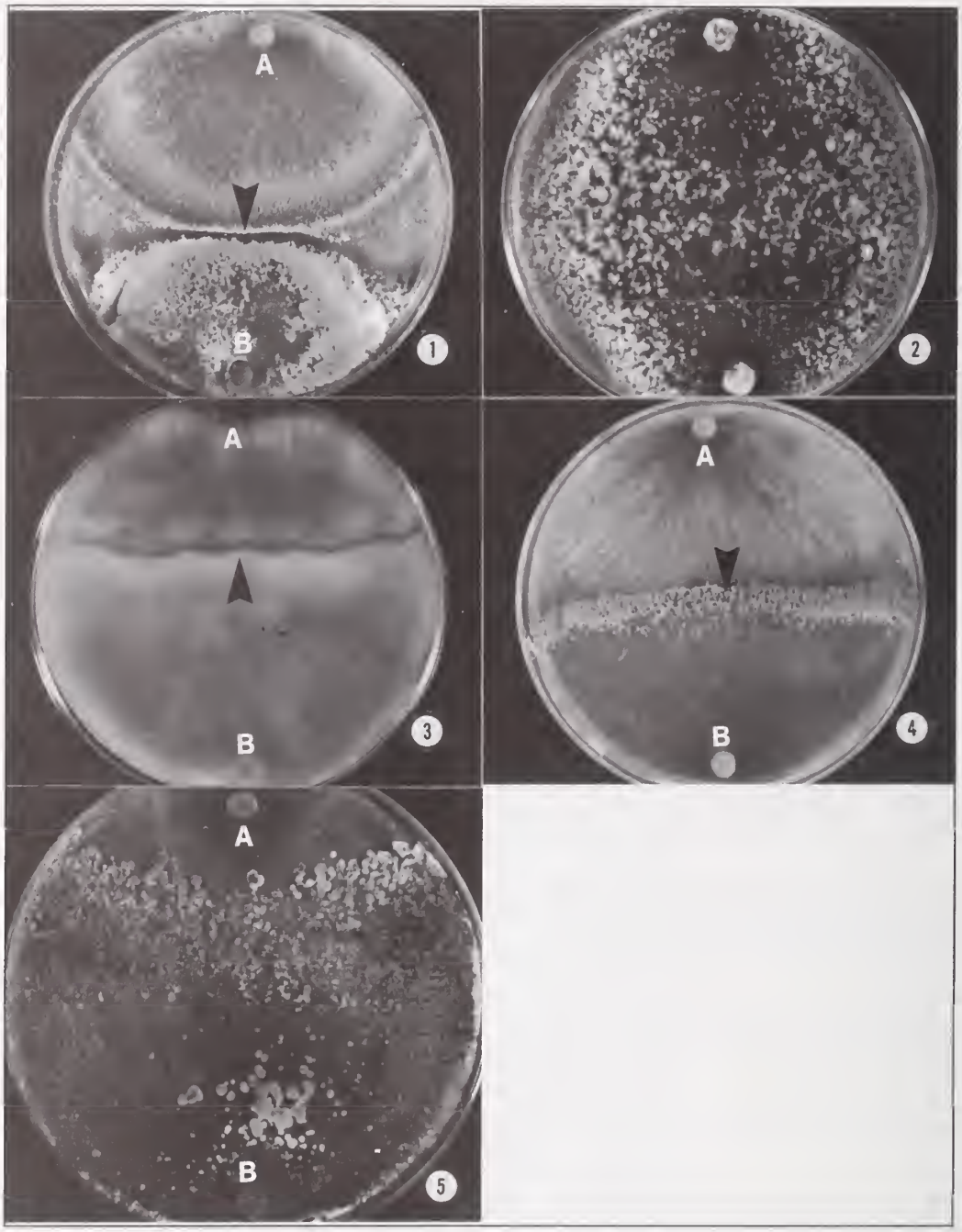
[2] Reaves, Jimmy L.; Crawford, Ralph H. Unpublished data. On file with: Forestry Sciences Laboratory, 3200, S.W. Jefferson Way, Corvallis, OR 97331.



Figures 1-5—Interactions appearing between colonies of *Trichoderma* grown in dual culture.

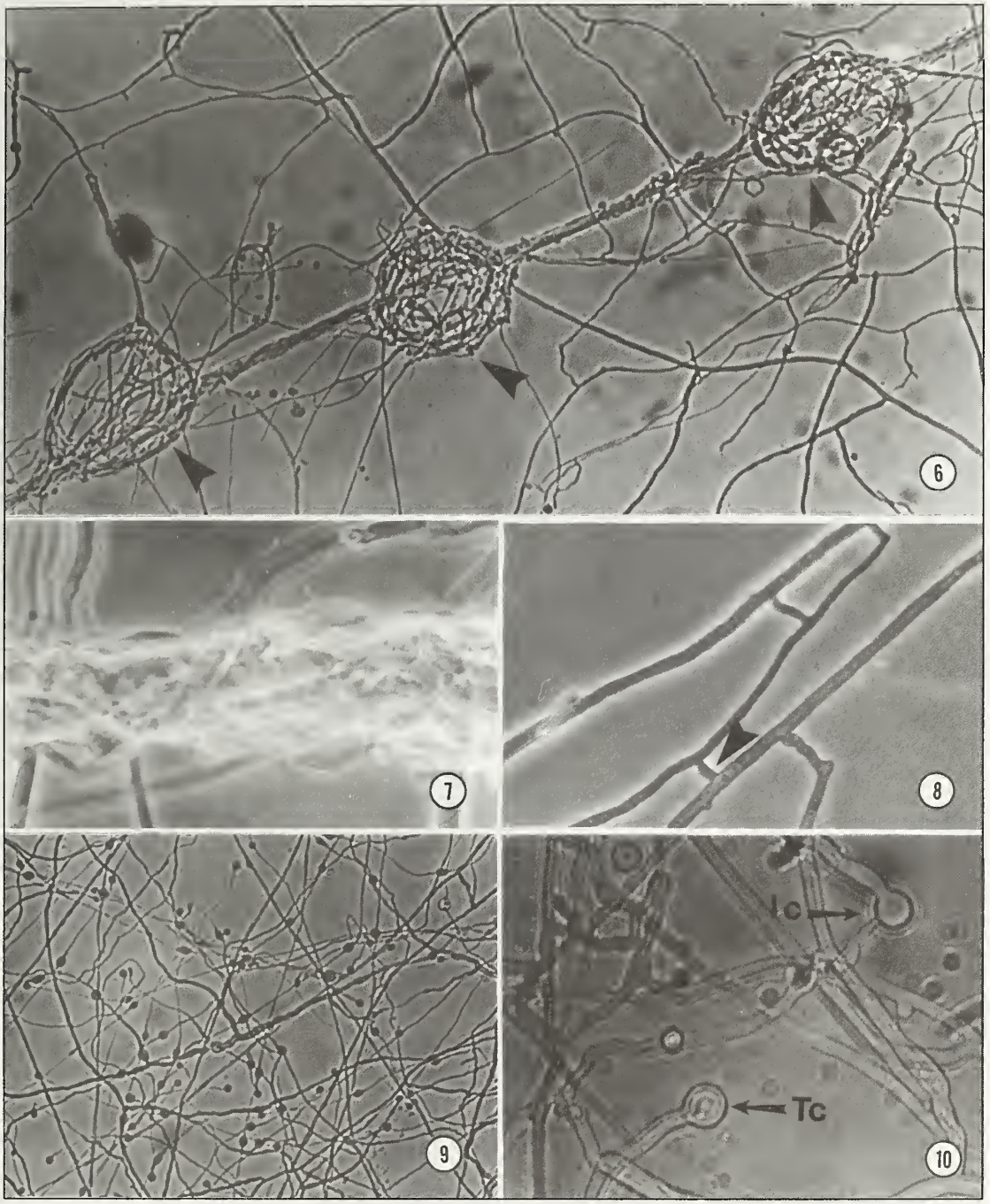
1—Zone of inhibition (arrowhead) between colonies of *T. hamatum* (A) and *T. citrinoviride* (B).

2—Demarcation line; pigmented line (arrowhead) that has formed in the medium between *T. viride* (A) and *T. longibrachiatum* (B).

3—Intermingling between self-pairing of one isolate of *T. hamatum*. The original zone disappeared and the mycelia grew together and appeared as one colony.

4—Ridge of conidia; accumulation of conidia (arrowhead) between colonies of *T. satumisporum* (A) and *T. longibrachiatum* (B).

5—Overgrowth; *T. citrinoviride* (A) has overgrown *T. longibrachiatum* (B) and sporulated.



Figures 6-10—Phase-contrast micrographs of hyphae in the interaction zones of paired isolates of *Trichoderma* spp.

6—Circular hyphal coils (arrowheads) formed at the interaction zone between *T. hamatum* and *T. longibrachiatum.* ×1,200.

7—A lateral coil in a paired culture of *T. viride* and *T. longibrachiatum.* ×1,200.

8—Anastomosis (arrowhead) of swelling hyphae in self-pairings of *T. hamatum.* ×1,200.

9—A mass of hyphae containing terminal and intercalary chlamydospores in paired cultures of *T. harzianum* and *T. saturnisporum.* ×200.

10—Enlarged terminal (Tc) and intercalary (lc) chlamydospores in paired cultures of *T. harzianum* and *T. saturnisporum.* ×1,400.

Table 1—Pairing interactions of isolates of *Trichoderma* spp. on malt agar

Interactions[a]	Parings[a]		
	Self	Intraspecific	Interspecific
	– – – – *Number[b] and percent[c]* – – – –		
Zone of inhibition	9 (23)	6 (33)	27 (12)
Demarcation line	0 (0)	0 (0)	48 (22)
Ridges of conidia	3 (7)	3 (17)	114 (53)
Overgrowth	0 (0)	0 (0)	27 (13)
Intermingling	27 (69)	9 (50)	0 (0)
Total pairings	39 (100)	18 (100)	216 (100)

[a] Definitions given in text.
[b] Number for any given interaction; N = 273 interactions.
[c] Values in parentheses are percentages of total pairings.

Table 2—Microscopic observations of self-pairings and intraspecific and interspecific pairings of species of *Trichoderma*

Interaction observations[a]	Pairings[a]		
	Self	Intraspecific	Interspecific
	– – – – *Number[b] and percent[c]* – – – –		
Coiling[d]	2 (15)	2 (33)	8 (11)
Anastomoses	11 (85)	3 (50)	0 (0)
Unaffected hyphae[e]	0 (0)	1 (17)	64 (89)
Total pairings	13 (100)	6 (100)	72 (100)

[a] Definitions given in text.
[b] One observation of each pairing; N = 91 interactions.
[c] Values in parentheses are percentages of total pairings.
[d] Circular coiling and lateral coiling are included in these values.
[e] Hyphae at the interaction zones between isolates that did not exhibit coiling or anastomosis.

Circular hyphal coiling occurred frequently in this study. Elad and others (1987) associated similar coiling with mycoparasitism of *Rhizoctonia solani* Kuhn by *T. harzianum* because such coils were not produced in single cultures of *T. harzianum*. Because hyphae of *Trichoderma* spp. are morphologically similar, it was impossible to determine if the circular coiling occurred between hyphae of the same isolate or hyphae of different isolates. However, because coiling (circular or lateral) was observed in the interaction zones and not in other portions of the colony in dual cultures, the evidence suggests that coiling resulted from hyphal interactions between colonies.

Chlamydospores were also observed microscopically. Lifshitz and others (1984) associated chlamydospore formation at the site of colony interaction between *Phythium nunn* Lifshitz, Stanghellini, and Baker and *T. koningii* Rifai with antagonism. The lack of chlamydospore production in single cultures and their abundance in the interaction zones in dual cultures suggests that they are stimulated by another competitor.

In vitro antagonism studies and microscopic examination should be considered in the evaluation of *Trichoderma* species when more than one species are used as potential biological control agents. An understanding of the compatibility between species or isolates of *Trichoderma* under various cultural conditions will provide information on the use of multiple species of *Trichoderma* as biological agents against a particular plant pathogen.

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Keywords: *Trichoderma* spp., biological control, antagonism.

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